Comparison of electrolyzed strong water acid and polyhexamethylene biguanide as wound cleansing solution to reduce bacterial colonization



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Wound cleansing solution is an essential modality in the treatment of Diabetic foot ulcer (DFU). The current study evaluated the effectiveness of electrolyzed strong water acid (ESWA) and polyhexamethylene biguanide (PHMB) as wound cleansing to reduce bacterial colonization. This was an experimental study which divided Wistar rats into three groups (ESWA, PHMB, and Control). The Kruskal-Wallis analysis confirmed that no difference of bacterial load but difference in wound size between groups (p <0.050). The results show that the role of ESWA as a wound cleansing solution to reduce bacterial load remains unanswered, but it suggests that ESWA has a positive effect in reducing wound diameter.

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Diabetic foot ulcer (DFU) is one of the complications of diabetes mellitus (DM), and the primary cause of amputation of the lower limb (Arya et al, 2014), which is supported by the current meta-analysis (Shin et al, 2017). If the DFU not adequately managed, it will become infected. The previous study confirmed the majority patient with DFU infected (Cervantes-García and Salazar-Schettino, 2017). This fact indicates the importance of optimizing wound care in DFU.

Wound cleansing is an essential part of wound care, creating an optimal wound environment by removing foreign objects, reducing bacterial counts and preventing biofilm activity on the wound surface. Various studies have been conducted to evaluate wound cleaning solutions such as propylbetaine-polihexanide (PP) and normal saline (Bellingeri et al, 2016), hypochlorous acid (Bongiovanni, 2016), sodium hypochlorite (NaClO) (Cardile et al, 2014). Currently, electrolyzed strong water acid (ESWA) has been introduced in healthcare setting, such as; cleaning dentinal tube (Arias-Moliz et al, 2014), peritoneal lavage (Kubota et al, 2014), and nasal irrigation (Jiang et al, 2016). Thus, we postulated ESWA has potential effect as a wound cleansing solution in DFU.

ESWA has a potentially beneficial effect on wound care. Although it has acid, ESWA considers being safety in tissues because it has low cytotoxicity (Kubota et al, 2014). Also, ESWA had a bactericidal effect similar to 5.25% NaClO (Sodium hypochlorite) (Cheng et al, 2016). It has advantages over other solutions such as normal saline and Electrolyzed alkaline water because it is bactericidal, fungicidal, and virucidal (Kubota et al, 2014; Tamaki et al, 2014; Ovissipour et al, 2015; Jiang et al, 2016). The physiological and chemical characteristics of ESWA include a pH of 2.3–2.7; an oxidative-reduction potential (ORP) of 1000–1100 mV; and dissolvability in oxygen with a concentration of 10–30 ppm or 1.2 mM as hypochlorous acid (Kiura et al, 2002).

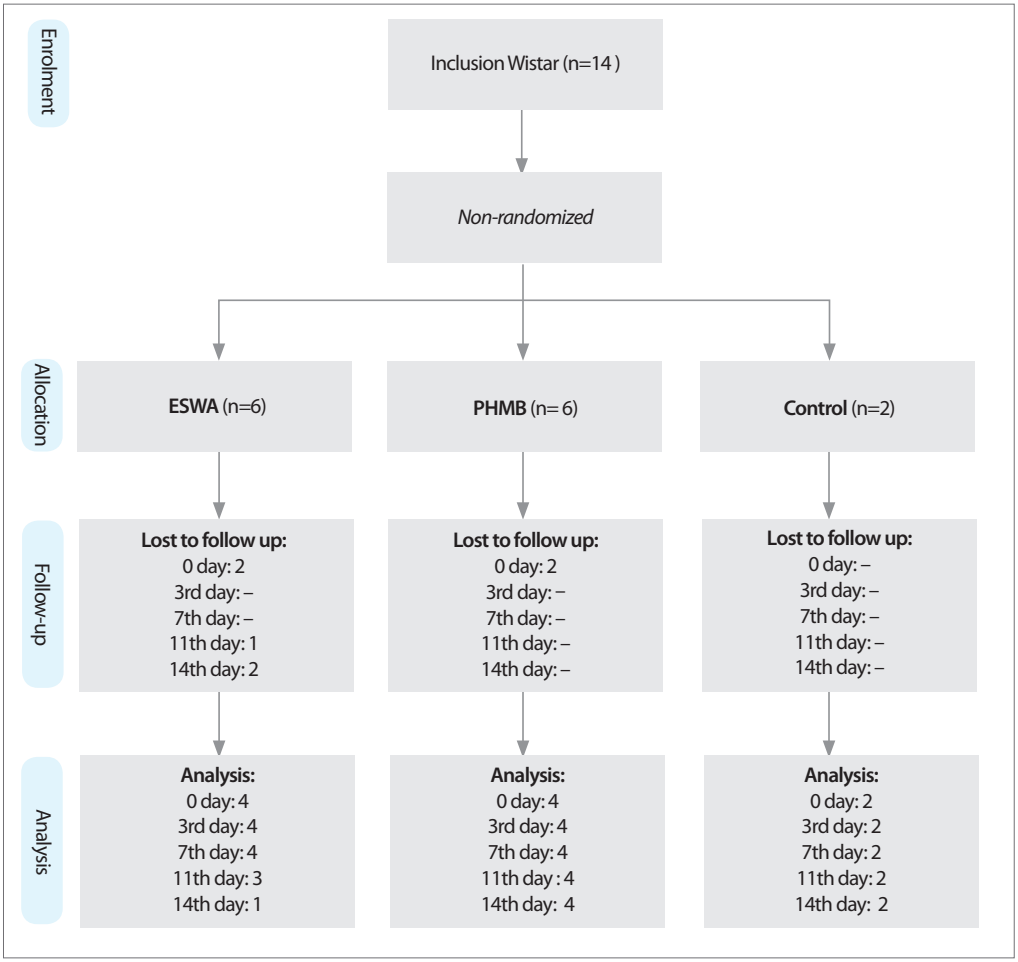
Although several studies evaluated the advantages of ESWA, the effectiveness of ESWA as a wound cleansing solution to reduce bacterial colonization remain unanswered. Thus, the aim of this study to compare the effectiveness of ESWA and PHMB as wound cleansing agents to reduce bacterial colonization in the wound healing process of the DM model Wistar.

Methods and procedures

Experimental animals

This was a quasi-experimental study using Wistar. Nine Wistars were allocated non-randomly into three groups: intervention group (cleaning with ESWA, n: 3), positive control group (cleaning with PHMB, n: 6) and negative control group (without treatment, n: 2). The weight of Wistar 250–300 gr (Kumar et al, 2013). DM was induced using 40–50 mg/kg/body weight in a single dose intraperitoneal injection of Streptozotocin (STZ) in a 0.1 M buffer citrate

Figure 1. ***Consort flow diagram***



solvent pH 4.5. Three days after the STZ injection each Wistar fasted for 4–6 hours (Deeds et al, 2011), and then blood glucose was measured in the tail vein (Lee and Goosens, 2015) using a Glucometer (Brăslaşu et al, 2007). The glucose level ≥250 mg/dl diagnosed as DM (Zangiabadi et al, 2011). All of Wistar provided by Animal Laboratory, Faculty of Medicine, Hasanuddin University, Indonesia.

The production of ESWA

The production of ESWA solution begins by flowing local mineral water into the ionized electrolysis water generator system (Kangen, Water Leveluk SD 501). First, water filtering from contaminants and harmful substances by the carbon filter inside the machine, after that, the water flow into the electrolysis chamber, and the water received a voltage of 230 watts which changes water structure. The output water, then measured by a pH meter. The presence of the pH water 2.5 defines as ESWA which is collected in a bottle and sterilized before its usage.

Experimental procedure

The Wistar's fur was removed using hair removal cream (Veet cream), and the skin disinfected with a 0.5% Chlorhexidine solution in 70% alcohol (Masoko et al, 2010). Inhalation anesthesia was carried out on DM Wistar rats with ether (Rao et al, 2007). The skin was anesthetized in the epidermis to the hypodermis/subcutaneous layer (to create a 2nd-degree wound) (Li et al, 2011), then a circular excision is made on the dorsal area to the deep fascia using an eight mm punch biopsy (Prestes et al, 2012). The area was dressed using low adherent dressing (Melolin, Smith & Nephew) and Adhesive tape (Hypapix, BSN Medical). The wound area was cleaned using ESWA (ESWA group). PHMB (PHMB group) and not cleaning for the control group. Reduction of colonization and wound size evaluated in 0, 3rd, 7th, 11th, and 14th days.

Bacterial load calculation

Wound swabs performed to collect bacterial samples for each observation day. Using the pour plate method, bacterial sampling dilutes with 1 ml NaCl, 0.9% in Eppendorf tube which diluted into seven Eppendorf tubes for each group. The bacterial dilution from Eppendorf

Table 1. Identification of the type of bacteria	
Group	**Type of bacteria**
ESWA	*Proteus vulgaris* *Staphylococcus aureus*
PHMB	*Proteus Mirabilis* *Proteus vulgaris* *Alkaligenes faecalis* *Staphylococcus aureus*
Control	*Staphylococcus aureus* *Proteus Mirabilis*

mixed with Plate Count Agar (PCA) centrifuged until homogenous on the petri dish. Following the solidification of the agar, the plate incubated for 18-48 hours at 37°C. The bacterial colony calculated as "Colony Forming Unit (CFU)."

Ethical clearance

The study protocol has received ethical approval from the Ethics Committee of the Faculty of Medicine, University of Hasanuddin Makassar, Indonesia number: 536 / H4.8.4.5.31 / PP36-KOMITE / 2018.

Results

Research flowchart

We included 14 Wistars which allocated non-randomly into three groups (ESWA, n: 6), (PHMB, n: 6) and (Control group, n: 2). During the period of study, we lost 2 Wistars at 0 days, one in 11th day, and 2 in 14th day (ESWA group). Also, we lost 2 Wistars in 0 days (PHMB group), and no one of Wistars lost in the control group *[Figure 1]*.

Types of bacteria

Our qualitative findings indicate the presence of *Proteus vulgaris* and *Staphylococcus aureus* in ESWA group. In PHMB group we identify *Proteus mirabilis, Proteus vulgaris, Alkaligenes faecalis,* and the presence of *Staphylococcus aureus*, while in the control group, we found both *Staphylococcus aureus* and *Proteus mirabilis [Table 1]*.

Reduction of bacterial load

Regarding of the bacterial load, in the ESWA group on day 0 was $7.5x10^8$ ($5.0x10^9$) and increased until the 11th day ($3.3x10^8$, ± SD $2.6x10^9$), then the bacterial load absence in 14th day. In PHMB group we found the absence of bacteria in 0 days and increased from the 3rd day ($7.9x10^7$, ± SD $1.5x10^9$) to be ($2.7x10^8$, ± SD $5.5x10^9$) at 11th day, but reduced in 14th day ($5.7x10^7$, ± SD $1.1x10^9$). In the Control group, bacterial increased during the first week ($2.0 x10^7$, ± SD $2.8x10^7$) to be ($1.5x10^8$, ± SD $2.1x10^9$), then reduced to be ($1.2 x10^8$, ± SD $1.7x10^9$) at the end of observation day. In general, we found, no difference of bacterial load between the different group, as confirmed by Kruskal-Wallis analysis *[Table 2]*.

Wound closure by the diameter

To evaluate wound healing, we measured wound diameter, which reflects the wound closure process during periods of observation. Macroscopically the wound diameter in each group decreased during the period of observation days. The wound size was decreased 3 mm both ESWA and PHM group within two weeks, meanwhile in the Control group reduction presence only 2 mm. The Kruskal-Wallis analysis confirmed that wound closure was significant between groups (p <0.050) *[Table 3]*.

Discussion

In this study, we found both gram-negative (-) and gram-positive (+) bacteria. The previous study confirmed that *Staphylococcus aureus* is the most common type of bacteria found in wounds, and significantly, is more frequent in

Table 2. Bacterial colonization (Mean, ± SD) between ESWA, PHMB, and Control group based on observation day

	Bacterial Number									
Group	**0 day (CFU/uL)**	***p***	**3rd day (CFU/uL)**	***p***	**7th day (CFU/uL)**	***p***	**11th day (CFU/uL)**	***p***	**14th day (CFU/uL)**	***p***
ESWA	$7.5x10^8$ ($5.0x10^9$)	0.086	$5.5 x10^7$ ($4.2x10^8$)	0.604	$1.0x10^8$ ($1.4x10^9$)	0.907	$3.3 x10^8$ ($2.6x10^9$)	0.271	0	0.214
PHMB	0		$7.9x10^7$ ($1.5x10^9$)		$1.4 x10^8$ ($1.4x10^9$)		$2.7x10^8$ ($5.5x10^9$)		$5.7x10^7$ ($1.2x10^9$)	
Control	$2.0x10^7$ ($2.8x10^7$)		$1.6x10^8$ ($2.0x10^9$)		$1.5x10^8$ ($2.1x10^9$)		$2.5 x10^7$ ($7.1 x10^7$)		$1.2x10^8$ ($1.7x10^9$)	

Table 3. Wound size (Mean, ± SD) reduction between ESWA, PHMB, and Control group based on observation day

Group	**0 day Mean ± SD (mm)**	***p***	**3rd day Mean ± SD (mm)**	***P***	**7th day Mean ± SD (mm)**	***p***	**11th day Mean ± SD (mm)**	***p***	**14th day Mean ± SD (mm)**	***p***
ESWA	8.0±0.0	1.000	7.5±0.6	0.259	6.8±0.5	0.472	5.7±0.6	0.700	5.0± NA	0.050
PHMB	8.0±0.0		7.3±0.5		6.5±0.6		6.5±0.6		5.0±0.0	
Control	8.0±0.0		8.0±0.0		7.0±0.0		6.0±0.0		6.0±0.0	

patients with chronic wounds (48.8%) than in patients with acute wounds (9.5%) (Wong et al, 2015). This study corresponded with previous research, which demonstrated that the dominant type of bacteria found in the three groups is *Staphylococcus aureus*.

The first question in this study sought to determine the effectiveness ESWA as a clean wound solution to reduce bacterial load. On the second week of observation, bacterial load reduced in ESWA but increased in PHMB and Control group. The log reduction of bacterial load 7.5x105 in ESWA group, but these data must be interpreted with caution because the PHMB group has increased (5.7 x 105) and Control group (11.9 x 105). The Kruskal-Wallis also confirmed that these results were not statistically significant. Despite ESWA reduce bacterial load (Cheng et al, 2016), with various advantages such as; including bactericidal, fungicidal, virucidal effect (Tamaki et al, 2014; Kubota et al, 2009; 2015; Ovissipour et al, 2015; Jiang et al, 2016), this study has been unable to demonstrate it. The possibilities of explanation of these results are due to variations of data (wider range standard of deviation) and small sample size.

The second question in this research was to evaluate the effectiveness of ESWA against the wound diameter reduction. In the current study, comparing ESWA and PHMB with Control showed that the mean wound diameter group has reduced in the third day and getting smaller at the end of observation day. However, although the wound sizes decreased over the observation period, the significant difference found only at 14th day. As suggested by previous studies, wound treatment with ESWA has an average reduction of 11% (Ricci, 2016). Thus, this finding indicates the potential beneficial effect of ESWA in the wound healing process.

Based on the results of this study, using ESWA as a wound cleansing solution is not very different from using PHMB. The role of ESWA in reducing bacterial load remains unanswered, but it has a positive effect in the wound healing process which needs more investigation.

Conclusion

The results of this investigation show that the role of ESWA as a wound cleansing solution to reduce bacterial load remain unanswered. However, it suggests that ESWA has a positive effect in reducing wound diameter. WAS

Disclosures

Financial disclosure:
All of the author states no conflict of interest in this study.

Studies involving animals:
This study has been adhered with animal welfare regulation and approval from the ethical committee from Faculty of Medicine, Hasanuddin University Makassar.

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